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Creating pathways towards aromatic building blocks and fine chemicals

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Aromatic compounds represent a broad class of chemicals with a range of industrial applications, all of which are conventionally derived from petroleum feedstocks. However, owing to a diversity of available pathway precursors along with natural and engineered enzyme 'parts', microbial cell factories can be engineered to create alternative, renewable routes to many of the same aromatic products. Drawing from the latest tools and strategies in metabolic engineering and synthetic biology, such efforts are becoming an increasingly systematic practice, while continued efforts promise to open new doors to an ever-expanding range and diversity of renewable chemical and material products. This short review will highlight recent and notable achievements related for the microbial production of aromatic chemicals.

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Introduction

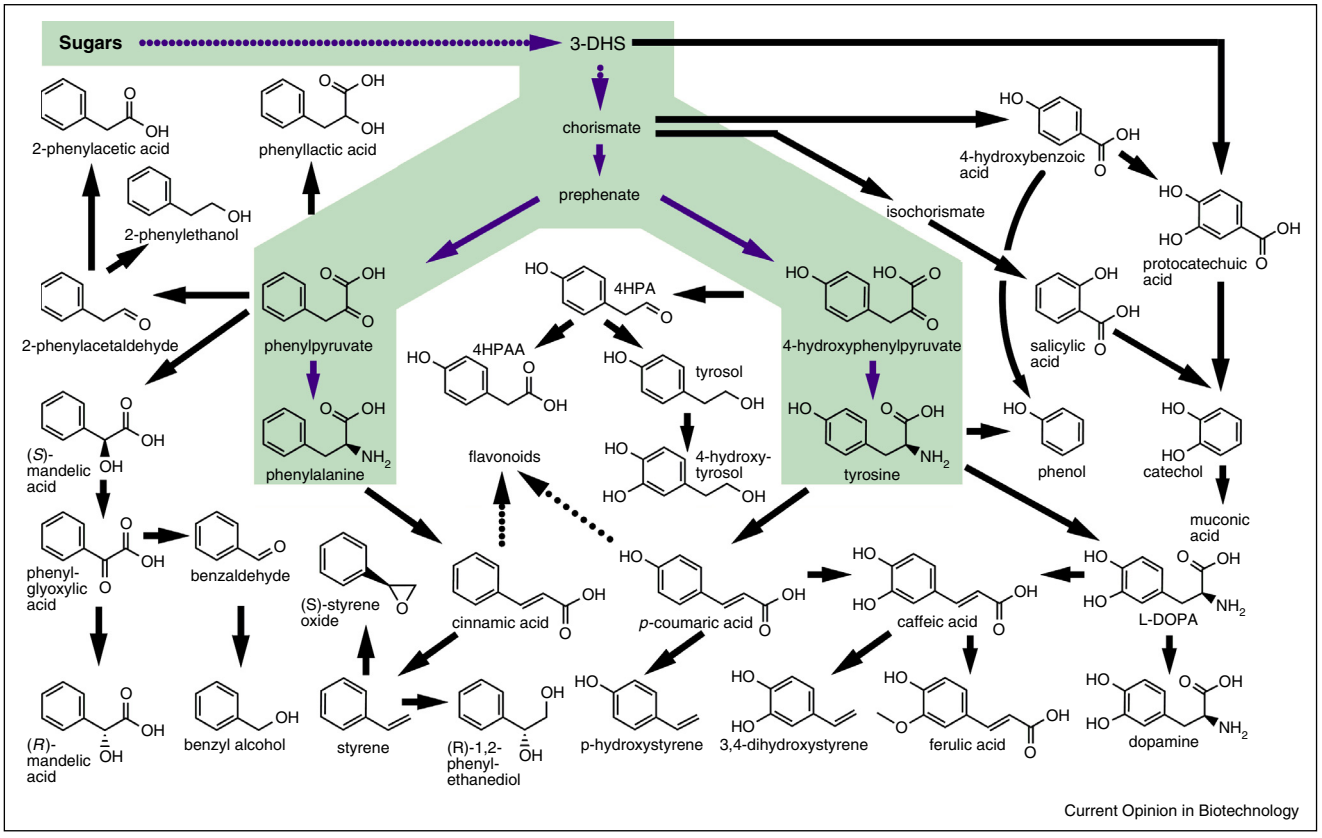
As the focus of many researchers in the biorenewables sector continues to transition from biofuels to biochemicals, the motivation behind the shift is clear: bulk and fine chemicals are of higher value than fuels (per unit mass) and serve significantly smaller markets (based on total carbon demand). Thus, although the total chemicals market utilizes only 5% as much carbon, the net economic value nearly equals that of fuels [1]. As a result, aggressive growth in this sector has been experienced worldwide in recent years — a trend that is expected to continue. In the United States, for example, production targets of bio-based chemicals and materials have been set at 18 and 25% of total production levels by 2020 and 2030, respectively [2]. In Europe, meanwhile, net chemical production from biomass is expected to reach 30% by 2030 (up from 10% in 2013) [3]. In addition to the continued development of established bioproducts, further growth and diversification amongst the renewable biochemicals market will occur most significantly through the engineering of novel microbial biocatalysts capable of synthesizing new and useful products as renewable replacements to conventional petrochemicals. Several recent examples illustrate how this approach has already been used to broaden the spectrum of chemical products that can be synthesized from renewable biomass, including 1,4-butanediol [4], iso-butanol [5], isoprene [6], and various organic acids [7–9].

Aromatic compounds represent another important class of conventional petrochemicals that have recently emerged as promising targets for metabolic pathway engineering. Conventionally derived from non-renewable petroleum feedstocks including benzene, toluene, and xylenes (BTX), aromatic chemicals serve a vast market and find extensive applications in the production of various solvents, plastics, and fine chemicals [10]. In 2010, for example, global annual consumption of benzene alone was estimated at over 40 million tons and growing steadily [11]. Using microbes such as *Escherichia coli* and *Saccharomyces cerevisiae* as hosts, novel enzyme pathways are alternatively being engineered to enable the novel biosynthesis of an increasing array of aromatic chemicals directly from renewable, biomass-derived feedstocks (Figure 1). In general, the novel ability of microbes to produce aromatic biochemicals has been accomplished through either (a) the functional reconstruction of natural-occurring pathways (typically from higher and/or less tractable organism; e.g., plants and fungi) or (b) *de novo* pathway engineering. Supported by advancements in bioinformatics, systems and synthetic biology, protein engineering, and 'omics' tools, the latter approach continues to emerge as an increasingly systematic practice, and one that is uniquely enabling the biosynthesis of non-natural aromatic chemicals. This short review aims to provide an overview of recent and impactful efforts to this end, with particular emphasis given to enabling factors uniquely associated with the biosynthesis of aromatic chemicals.

A diverse precursor pool supports a range of aromatic products

With few exceptions (perhaps only phloroglucinol, a phenolic compound derived directly from malonyl-CoA [12]), aromatic pathways have been constructed by utilizing endogenous precursors derived from the ubiquitous shikimate pathway. Accordingly, achieving appreciable

Figure 1



By applying metabolic engineering and synthetic biology tools, researchers have engineered a collective 'network' of biosynthetic pathways to enable the microbial biosynthesis of an array of useful aromatic chemicals from renewable, biomass-derived sugars. Illustrated are both functionally reconstructed, naturally occurring pathways as well as novel, non-natural pathways. Shaded regions depict endogenous metabolism. *Note*: Trp derived pathways are excluded for clarity and space.

production of any aromatic target first requires metabolite flux to be enhanced through this otherwise tightly regulated pathway: a prerequisite condition that has been met in numerous microbial platforms, typically as a result of both combinatorial (e.g., metabolic evolution) and rational approaches [13•,14,15•]. A key and relatively unique factor enabling access to a broad range of established and putative final aromatic products is in fact the diverse pool of uniquely functionalized metabolites that can serve as pathway precursors (Figure 1) — namely those between 3-dehydroshikimate (3-DHS) and the terminal aromatic amino acid (AAA) products (i.e., tyrosine (Tyr), phenylalanine (Phe), and tryptophan (Trp)). Lying at a highly regulated metabolic node, chorismate serves as a particularly useful precursor to numerous aromatic pathways. Meanwhile, with conserved 4-hydroxyl functionality, phenolic products have typically been derived from the Tyr branch, including phenol [16], *p*-hydroxybenzoate [17•], coumaric acid [18], caffeic acid [19,20], ferulic acid [20], *p*-hydroxystyrene [21], tyrosol [22], and hydroxytyrosol [23]. Stemming from the Phe branch, pathways have been engineered to 2-phenylethanol [24,25••], cinnamic acid [18], styrene [14,26], (*S*)-styrene oxide [27], (*R*)-1,2-phenylethanediol [27], (*S*)-mandelic and (*R*)-mandelic acid [28], and benzaldehyde [29•]. Although comparatively fewer routes have to date been constructed from the Trp branch, notable examples include indigo dye [30] and catechol (though subsequently converted to muconic acid) [31].

Constructing aromatic biosynthesis pathways from robust enzyme 'parts'

With a vast and comprehensive 'toolkit' of known and/or putative enzyme chemistries — including those with high chemo-specificity, regio-specificity, and/or stereo-specificity — efficient construction of aromatic biosynthetic pathways is further supported by the availability of an abundance of useful enzyme 'parts'. In this case, inherent metabolic diversity draws from both anabolic and catabolic processes, co-evolved predominantly as a result of plant–microbe interactions. Within the rhizosphere and throughout the soil environment, for example, secondary aromatic metabolites synthesized by plant pathways (e.g., phenolic signaling and defense molecules,